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Interaction of MALDI matrix molecules with Na^+ in the gas phase

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Dedicated to Dr. Yannik Hoppilliard, a very special person in addition to a great colleague.

Abstract

The sodium complexes of six molecules commonly used as matrices for matrix-assisted laser desorption ionization (MALDI) have been investigated by ab initio calculations. Several isomers have been considered in each case, including salt bridges. In one case, the sodium complex of nicotinic acid (NA), the lowest energy isomer is a salt bridge. The adequate computational level has been established through calibration on the sodium complex of 2,5-dihydroxybenzoic acid (DHB). Accurate sodium ion binding enthalpies have been derived for DHB (34.5 kcal/mol), anthranillic acid (37.0 kcal/mol), NA (37.5 kcal/mol), sinapinic acid (39.0 kcal/mol), picolinic acid (42.9 kcal/mol), and 4-OH-α-cyanocinnamic acid (44.3 kcal/mol). Some of these values can be compared to recent experimental measurements. Their relevance to the interpretation of secondary MALDI processes in the plume is discussed. (Int J Mass Spectrom 219 (2002) 577–592)

Keywords: Sodium affinity; MALDI matrix; Ab initio calculations; Gas phase

1. Introduction

Interaction of sodium cation with organic molecules is interesting in a variety of contexts. In the condensed phase, the sodium cation is often used as a Lewis acid catalyst for electrophilic assistance to organic reactions such as nucleophilic additions to multiple bonds. The mobility of sodium ions into zeolithe channels or into ion transmembrane channels of cells, which is governed by local interactions of Na^+ with various types of inorganic and organic sites, respectively, is also of great importance. The fundamentals of such interactions can be learned from gas phase studies, both experimental and theoretical. In the recent past, methods have been developed to generate accurate sodium ion affinities of a variety of molecules. A large body of data has been acquired, and several compilations are now available [1–3]. There have been three main experimental methods used for obtaining accurate affinities. These include pulsed high pressure mass spectrometry [1], low pressure ligand exchange equilibria [2], and guided ion beam mass spectrometry [3]. The kinetic method has also been shown to be very useful [4]. In all cases, the complementarity of experimental and computational studies has been essential. In fact, the types of interactions involved in the complexes of the sodium cation with organic/biological molecules are relatively easy to calculate with standard quantum chemical methods, so that computation now provides the easiest access

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to accurate sodium ion affinities of molecules. This is especially true after careful analyses of the influence of computational level on affinity accuracy [5,6].

Matrix-assisted laser desorption ionization (MALDI) of molecules has become a very powerful and widely used tool for obtaining gaseous ionized molecules which are too fragile to be brought to the gas phase by some of the other techniques available [7]. The complexation by alkali metal cations such as sodium is interesting since it provides an entry into the gaseous state without a strong perturbation of the neutral molecule. It is also interesting since it has been shown, e.g., for peptides, that alkali ion cationized species fragment differently from their protonated analogues (see, e.g., [8]). MALDI matrices are known to have rather different propensities toward the production of sodium cationized molecules [9]. Although the mechanisms of ion formation are only partly understood, there is converging evidence that the primary events following photon interaction with the solid sample cannot suffice to account for the identity and distribution of ions eventually detected in the mass spectrometer. It is likely that secondary events occurring in the 'plume' have a strong influence on the observed spectra. Processes such as reduction by free electrons, charge transfer, excited state proton transfer and metal ligand exchange, inter alia, have been invoked recently with some compelling evidence [10,11]. The formation of sodiated molecules might, at least in part, arise from sodium transfer reactions in the gas phase [11]. It is therefore, important to know the gas phase sodium affinity of MALDI matrix molecules with good accuracy to evaluate how much such reactions participate in the MALDI process. At least such values may be viewed as some of the necessary parameters to input into the rather complex models of MALDI which are currently emerging.

2. Computational

The calculations of binding energies were carried out following procedures which have been described and used previously. Geometries for the bare neutral and sodium cationized molecules were determined by full optimization followed by a vibrational frequency analysis. This was generally done at the Hartree–Fock level using the standard 6-31G∗ basis set, denoted as HF/6-31G∗ or HF/b1 herein. Final energetics were obtained at the HF/6-31G∗ geometries via energy calculations at the MP2 level with no frozen core, using the 6-311+G(2d,2p) basis set (labeled as b2). This level is denoted herein MP2/b2//HF/b1. Previous work has shown that sodium binding energies of various small molecules computed at this MP2 level are accurate, probably due to a cancellation of (small) errors [5]. Based on these results, no basis set superposition error (BSSE) correction was applied to the results. Zero-point vibration energies (ZPVE), and thermal corrections at 298 K, were obtained from the vibrational analysis and used to obtain 0 and 298 K binding energies, respectively. Binding entropies were also obtained to derive binding free energies.

The sodium complex of 2,5-dihydroxybenzoic acid (DHB) was used to compare several computational procedures. Previous work has shown the high accuracy of the MP2/b2//MP2/b1 level, however, optimizing geometries and especially carrying out vibrational analyses at the MP2 level is resource demanding, an undesirable feature in the prospect of extending this work to significantly larger molecules. Therefore, HF/b1 and MP2/b1 geometries were compared, both with final energetics at the MP2/b2 level. Their results were further checked against a still higher level binding energy, which was obtained by extending the basis set up to aug-cc-pVTZ for H, C and O and to aug-cc-pCVTZ for Na [12], using the MP2/b1 geometries. This reference level is denoted as MP2/b3//MP2/b1. It has been shown to yield results nearly identical to those obtained by CCSD(T) computations with large basis sets [5], a level beyond reach for molecules of the size considered in the present work. Finally, the B3LYP and MPW1PW91 density functionals were also compared, with geometry optimizations and vibrational frequency calculations using the 6-31+G∗ basis. Since basis set dependence is generally much smaller with density functionals than with ab initio wavefunctions such as HF or MP2,

energetics were obtained with the same basis set in this case. All calculations used five-component d sets and seven-component f sets.

As is well documented in the literature, vibrational frequencies obtained at the HF/6-31G∗ level are overestimated, and a scaling factor of ca. 0.91 has been recommended for calculating ZPVE and thermal corrections. The average error is much smaller with MP2/6-31G∗ and B3LYP/6-31+G∗ wavefunctions, with recommended scaling factors of 0.96 and 0.98, respectively. Here however, we consider binding energies which are differences of absolute energies. Therefore, there is an extensive cancellation of errors, and the use of a scaling factor is insignificant. For instance, the ZPVE plus 298 K correction to the DHB–Na^+ binding energy is computed to be 0.9, 1.1 and 0.9 kcal/mol at the HF/6-31G∗, B3LYP/6-31+G∗ and MP2/6-31G∗ levels, respectively.

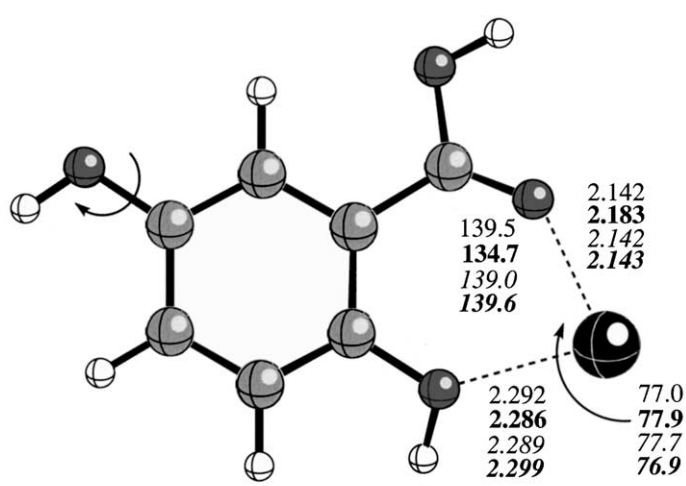
Binding energies were computed as the energy difference between isolated species (neutral molecule and sodium cation) on the one hand and the sodium complex on the other, each in its most stable structure. In most cases this required considering several conformations of the neutral molecule and several ion attachment sites for the complex. Binding energies were obtained as this energy difference even in cases where the bare and sodiated molecules are in different conformations, such as those related to each other by a cis-trans isomerization of the carboxyl group.

All computations were carried out using the Gaussian 98 package [13].

3. Results

3.1. DHB

An extensive search of the potential energy surface was carried out for the sodium complex of DHB. Some of the resulting minima are displayed on Figs. 1 and 2. The lowest energy structure was found to involve Na^+ chelation between the carbonyl oxygen of the acid and the 2-hydroxy group. Two nearly equivalent structures (labeled **1** and **2**) are generated by the two possible conformations of the 5-OH group in the molecular plane. Only one is shown as **1** in Fig. 1,



DHB-Na^+ **1**

Fig. 1. Optimized geometry for the lowest energy isomer **1** of DHB–Na^+ at the HF/6-31G∗, MP2/6-31G∗ (bold), B3LYP/6-31+G∗ (italics) and MPW1PW91/6-31+G∗ (bold italics) levels. Distances are in Angströms and angles in degrees.

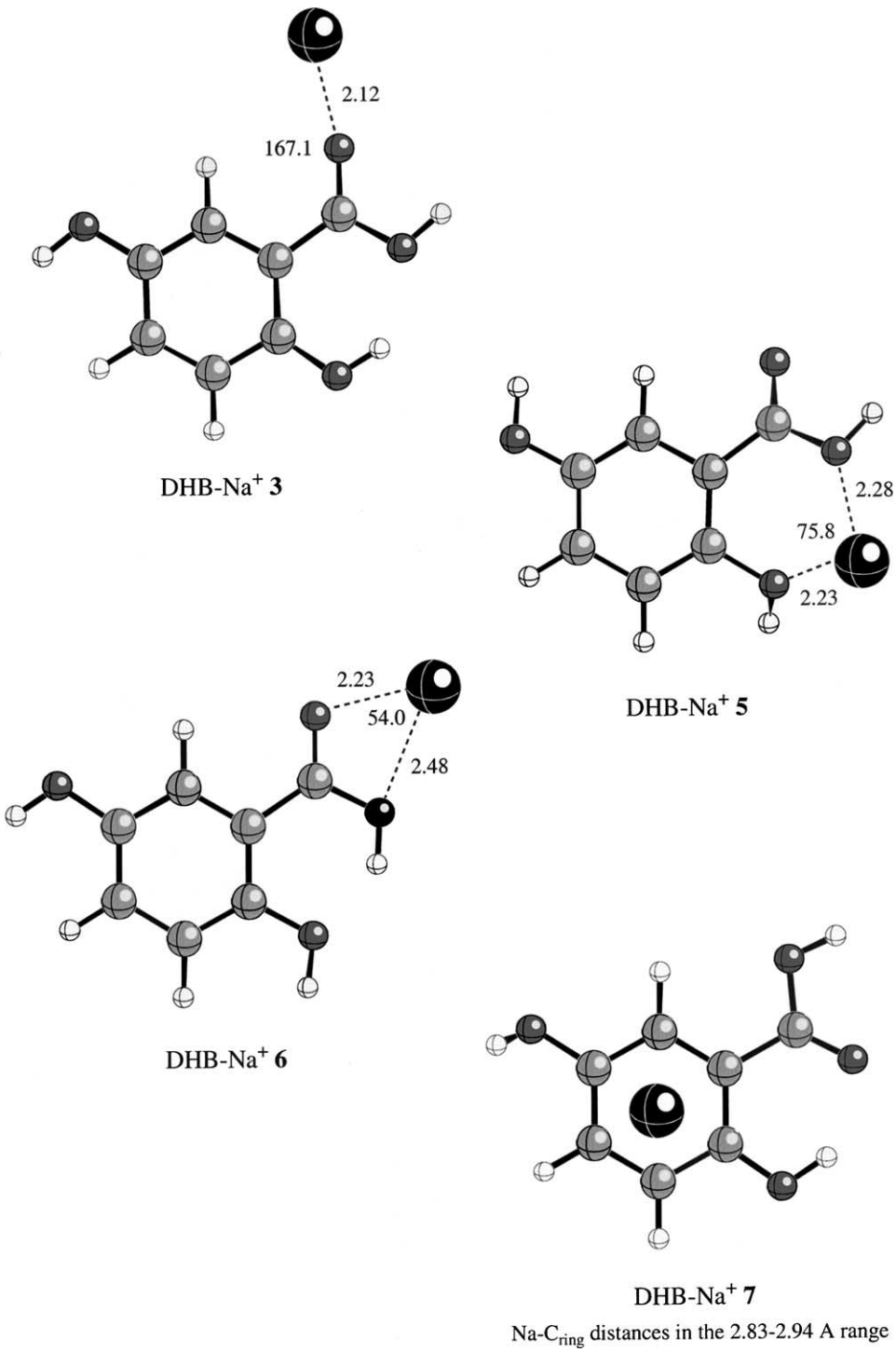


Fig. 2. Optimized geometries for selected isomers of DHB–Na^+ at the HF/6-31G* level. Distances are in Angströms and angles in degrees.

while **2** is obtained from **1** by a 180° rotation around the C–O bond indicated with an arrow in Fig. 1. The next most stable isomer (see **6** in Fig. 2) involves complexation of Na^+ with the carboxyl group in its trans conformation, enabling interaction with both oxygens. The trans conformation of a carboxylic acid being intrinsically unfavorable by ca. 8 kcal/mol relative to the cis, sodium complexation cannot compensate for this energy penalty. Attachment only to the carbonyl oxygen of the cis acid (see **3** in Fig. 2) is significantly less favorable, since Na^+ now interacts also with a neighboring hydrogen which carries a partial positive charge. If the H bond in **3**, between the 2-OH and carboxyl OH is broken, an isomer **4** (not shown) is formed which is 1.9 kcal/mol less stable than **3**. Another possibility involves chelation of Na^+ between the hydroxy oxygen and the 2-hydroxy group (see **5** in Fig. 2), which is slightly less favorable than **4**. Yet another isomer found is a π complex in which the sodium ion sits on top of the ring and interacts with the 5-OH group in which the hydrogen is bent out of plane (see **7** in Fig. 2). This enables electrostatic stabilization by interaction of the sodium charge with both the ring quadrupole and the OH dipole, in a manner which strongly resembles that found previously in the sodium complex of phenol [1]. Such isomers with cation–π interactions are know to be stabilized by charge-quadrupole as well as charge-induced dipole interactions [14]. However, the quadrupole moment of a phenyl ring is strongly reduced by electronegative substituents, so that other attachment sites are much more favorable in DHB. Finally, several attempts were made to locate salt bridge isomers in which the sodium ion would interact with the carboxylate group of DHB. The first involved the deprotonated carboxyl group H bonded to a protonated 2-OH group. This collapsed directly to isomer **5** by a simple proton transfer from the 2-OH to the carboxylate. The initial energies of this attempt were high enough that the existence of such a stable salt bridge isomer of this type appears to be unlikely. Another salt bridge can be formed by protonation of the 5-OH group. This isomer **8** (not shown) was found to lie 44.3 kcal/mol higher than **1**. This was, by far, the least stable of all structures investigated.

The optimized geometry of isomer **1** of DHB–Na^+ with four different wavefunctions is shown in Fig. 1. It is obvious from these results that all wavefunctions provide a satisfactory account of Na^+ attachment. One feature which is not easily apparent from Fig. 1 is that there is some out of plane distortion near the sodium ion in ab initio geometries, while DFT geometries are nearly exactly planar. This effect has little influence on energetics, since this out of plane motion is associated with a very small vibrational frequency for all wavefunctions.

The sodium affinity results for DHB–Na^+ **1** are summarized in Table 1. It can be seen, from the comparison with more accurate calculations, that both HF/b1 and MP2/b1 computed affinities are overestimated to an extent which makes them inadequate. This has already been detailed in a study of Na^+ complexes of small molecules [15]. The insufficient flexibility of the b1 basis cannot account for the electronic adjustment of the cation and of the molecule, leading to a small underestimation of the binding energy, while the large BSSE leads to a large overestimation. The combined effect of these contradictory errors leads to a significant overestimation of the binding energy of ca. 5–6 kcal/mol. On the other hand, the errors on the vibrational analysis (with frequencies significantly overestimated at the HF level) lead to negligible errors on the binding energy since errors made on the complex and on the free molecule are nearly exactly the same. The MP2/b2 values are very similar at the HF/b1 and MP2/b1 geometries. It may

Table 1
Calibration study on the binding enthalpy and free energy of Na^+ to DHB in isomer **1** of DHB–Na^+

Wavefunction	ΔH_{298}	ΔG_{298}
HF/b1	39.7	33.0
MP2/b1	40.2	33.1
B3LYP/6-31+G*	36.0	28.8
MPW1PW91/6-31+G*	34.5	27.4
MP2/b2//HF/b1	34.5	27.8
MP2/b2//MP2/b1	33.9	26.8
MP2/b3//MP2/b1	34.2	27.1

ΔH_{298} adds a work term of RT (0.6 kcal/mol at 298 K) to the variation of internal energy at this temperature. All values are in kilocalories per mole.

be concluded that for this type of molecule, HF/b1 is a satisfactory level for geometry optimization and vibrational analysis. The fact that the MP2/b2//MP2/b1 value is slightly smaller may not be used to deduce a systematic correction to MP2/b2//HF/b1 values for other cases, since the highest level used in this study, MP2/b3//MP2/b1 falls in between them. Based on the study by Feller [5] on the ethylene and benzene complexes of Na^+, we take the latter level as our accuracy reference. Since the MP2/b2//HF/b1 sodium affinity is found to be larger than this reference value by only 0.3 kcal/mol, we conclude that it provides a very efficient compromise between computational demand and affinity accuracy. Finally, DFT values can also be compared to this reference. As previously described for smaller molecules [15], the B3LYP functional yields binding enthalpies that are slightly too large. On the other hand, the MPW1PW91 functional yielded results in very good agreement with the best MP2 level. However, the computational demand is not so much lighter with this functional than it is with MP2, for which we have a large body of data available from previous work. Therefore, DFT computations were not pursued further in this study, but the MPW1PW91 functional might become the method of choice for larger cases.

Now that we know that the MP2/b2//HF/b1 approach is able to yield an accurate sodium ion affinity of DHB, there remains to consider the relative energetics of the various isomers of DHB–Na^+. Table 2 summarizes results obtained at the HF/b1 and MP2/b2//HF/b1 levels. It can be seen that although the HF/b1 values are not very accurate, there is no large difference between both set of values. The energy order may differ only in cases where differences are very small. We can therefore, conclude that the potential energy surface may be first explored at the HF/b1 level, and that only the lowest isomer be further calculated at the MP2/b2//HF/b1 level, together with other(s) isomer(s) with very small energy differences if any. This is the strategy used for all other cases in this paper.

Table 2
Relative energies of DHB–Na^+ isomers, including ZPVE and thermal corrections at 298 K

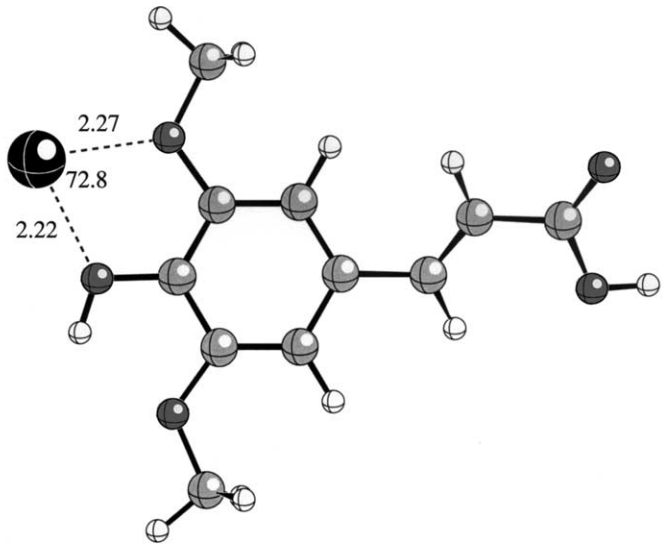
Isomer	HF/b1	MP2/b2//HF/b1
1	0	0
2	−0.1	0
3	9.7	8.6
4	11.6	10.4
5	12.7	10.2
6	2.9	0.7
7	18.6	13.9
8	44.3	–

The sodium ion affinity of DHB is computed to be 34.5 kcal/mol. This is the binding enthalpy at 298 K, in which the lowest energy isomer of DHB–Na^+ and the lowest energy conformation of free DHB are considered. The corresponding binding free energy is 27.8 kcal/mol. This value will be compared to experiment [16] in Section 4.2.

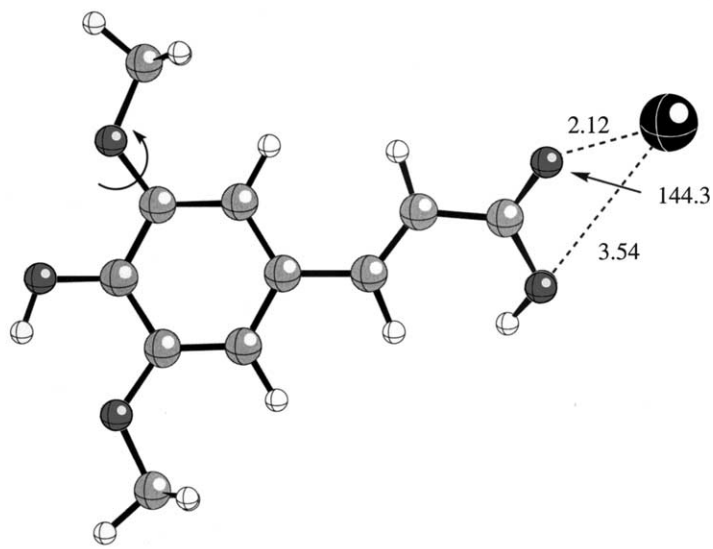
3.2. Sinapinic acid (SA)

There are clearly two competing modes of attachment of Na^+ to SA. One is by chelation between the 4-OH and either of the 3- or 5-methoxy groups. The second is on the carboxyl group, preferably in its trans conformation to enable interaction of the ion with both oxygens, as seen above for DHB.

The first type of isomer is depicted as **1** on top of Fig. 3. In this 1,4 chelation, Na^+ can accommodate two distances to oxygens which are both close to that to a single functional group, making this chelation efficient. This is in fact the lowest energy isomer found. Binding to the trans carboxyl is less favorable (see Table 3). As can be seen on the lower part of Fig. 3 (isomer **2**), the ion binds to the oxygens in a rather nonsymmetric fashion, contrary to what was found above in isomer **5** of DHB–Na^+. This is due to hydrogen–hydrogen repulsion between the carboxyl OH and the second olefinic CH. This forces the OH out of the molecular plane, and even with this distortion the nonbonded H–H distance is short, 1.98 Å. The out of plane OH dipole cannot interact as favorably with the sodium charge as in DHB–Na^+ **6**, and a better compromise is obtained by a stronger interaction of the ion with the carbonyl oxygen, at a distance which is shorter than in DHB–Na^+ **6** by



SA-Na^+ **1**



SA-Na^+ **2**

Fig. 3. Optimized geometries for isomers of the Na^+ complex of SA at the HF/6-31G* level. Distances are in Angströms and angles in degrees.

ca. 0.1 Å. A third isomer **3** (not shown) was found, very similar to **2**, in which the methoxy group which is not H bonded to the 4-OH is rotated out of the molecular plane by ca. 70° (see arrow on **2** in Fig. 3). At the HF/b1 level, it is slightly more stable than **2**, by 1.3 kcal/mol. Since it is still less stable than **1** by 7.5 kcal/mol at the HF/b1 level, only **1** was further considered for refined energetics.

Table 3
Relative energies at 298 K of the isomers of sodiated matrix molecules (except DHB, see Table 2), at the HF/b1 level

Species	Relative energy
SA–Na^+ **1**	0
SA–Na^+ **2**	8.8
SA–Na^+ **3**	7.5
4-HCCA–Na^+ **1**	0
4-HCCA–Na^+ **2**	11.9
4-HCCA–Na^+ **3**	29.4
PA–Na^+ **1**	0
PA–Na^+ **2**	6.8
PA–Na^+ **3**	3.3
NA–Na^+ **1**	9.7
NA–Na^+ **2**	10.8
NA–Na^+ **3**	0
AA–Na^+ **1**	0
AA–Na^+ **2**	7.9
AA–Na^+ **3**	7.0

All values are in kilocalories per mole.

Given the DHB results and the low basicity of the hydroxy and methoxy substituents, it was not expected that there would exist a salt bridge isomer of low energy. Indeed, proton transfer from the acid to the 4-OH group resulted in structures (not shown) lying about 40 kcal/mol higher than **1**.

The most stable conformation found for free SA is the same as that in the sodium complex **1**. Another conformation was found, in which the upper methoxy group is oriented towards the hydroxy, and in which the second methoxy group (to which the hydroxy is H bound) is rotated out of plane around its C–OMe bond. It is slightly more stable at the HF/b1 level, but slightly less stable at the MP2/b2//HF/b1 level. Finally, a third conformation involving a trans acid as in SA–Na^+ **2** was located 8.7 kcal/mol above the most stable one.

The binding enthalpy of Na^+ to SA, involving the lowest energy isomer SA–Na^+ **1** and the structurally similar, most stable conformer of free SA, is computed to be 39.0 kcal/mol.

3.3. 4-OH-α-cyanocinnamic acid (4-HCCA)

Three isomers were obtained for 4-HCCA–Na^+ (see Fig. 4). The most stable **1** involves sodium chelation between the carbonyl oxygen of the cis acid, and the nitrogen atom. The Na–N and Na–O lengths are close to their optimum values, but the ion cannot adopt a nearly optimum position relative to the local dipole moment of either group. In the other two isomers, the sodium ion is bound to the acid. In **2**, it interacts with both oxygens of the acid, while in **3** it interacts with both oxygens of the carboxylate while the cyano group is protonated. The energies of **2** and **3** are 11.9 and 29.4 kcal/mol higher than that of **1**, respectively. It is likely that the salt bridge structure **3** is a minimum despite its high energy only because the protonated cyano group is not oriented in a way that is favorable for proton transfer to the carboxylate, which would lead to the much more stable isomer **2**. The most stable conformation of free 4-HCCA is similar to the one in 4-HCCA–Na^+ **1**. The trans acid with a weak hydrogen bond to the cyano group as in 4-HCCA–Na^+ **2** lies 3.2 kcal/mol higher in energy at the HF/b1 level.

The binding enthalpy of Na^+ to 4-HCCA, involving the lowest energy isomer 4-HCCA–Na^+ **1** and the structurally similar, most stable conformer of free 4-HCCA, is computed to be 44.3 kcal/mol. This is the largest sodium binding enthalpy among those determined in the present work.

3.4. Picolinic acid (PA)

Three structures were determined for the sodium complex of PA. It seems most natural to chelate the sodium ion between the carbonyl oxygen and the ring nitrogen, as formation of this pseudo five-membered ring permits strong interaction of the ion on both sides (see **1** in Fig. 5). However, another alternative turned out to be nearly as stable as **1**: a salt bridge in which Na^+ is bound to the carboxylate, with the ring nitrogen being protonated (see **3** in Fig. 5). This structure is only 3.3 and 1.4 kcal/mol less stable than **1** at the HF/b1 and MP2/b2//HF/b1 levels, respectively. Contrary to the case of 4-HCCA, the 'neutral' isomer **2** generated from **3** by proton transfer from the nitrogen to the carboxylate is significantly less stable than the salt bridge, lying 3.5 kcal/mol higher than **3** at the HF/b1 level.

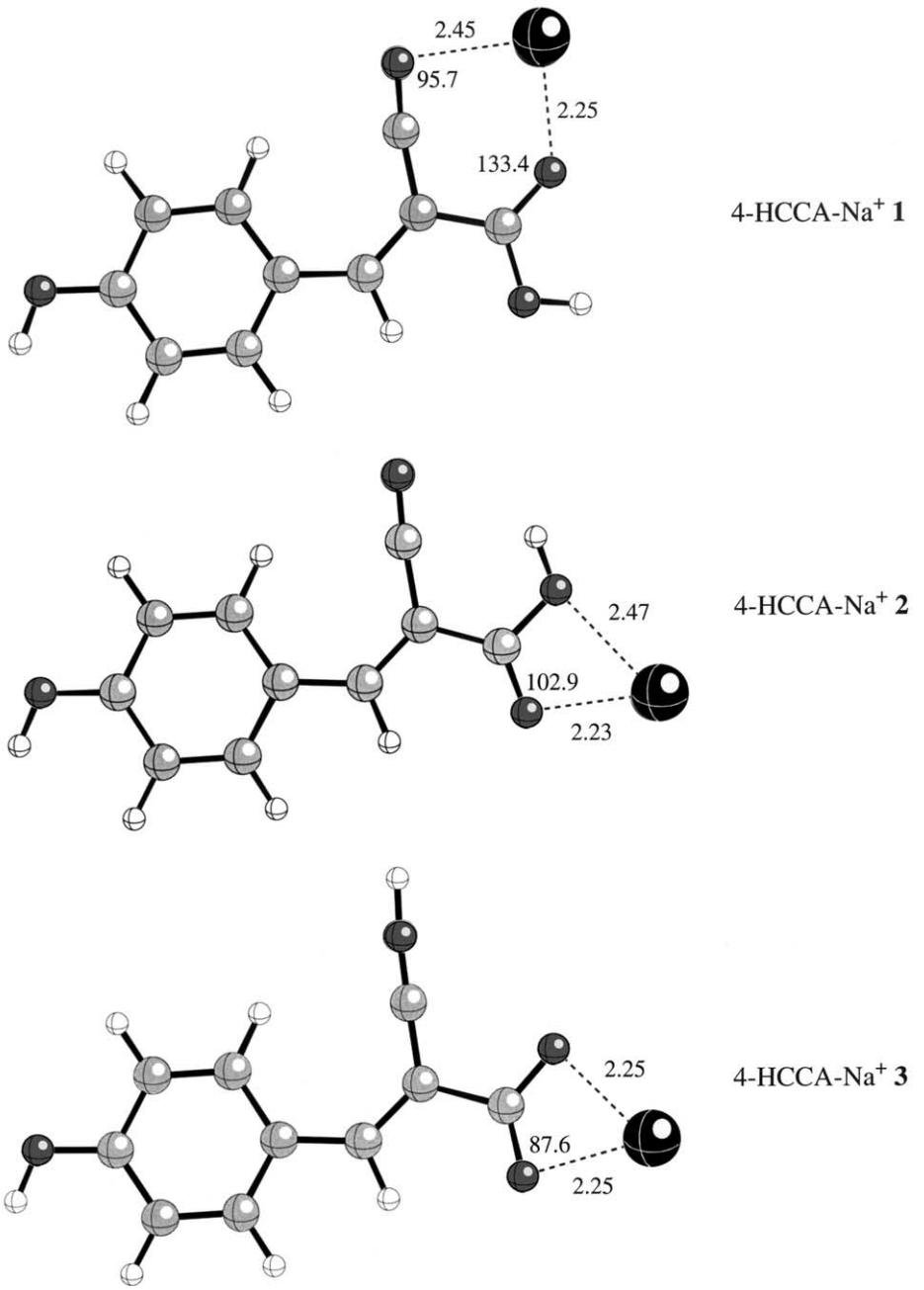
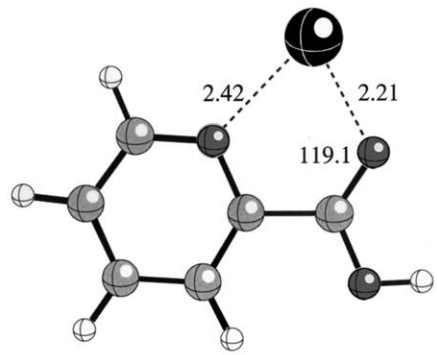
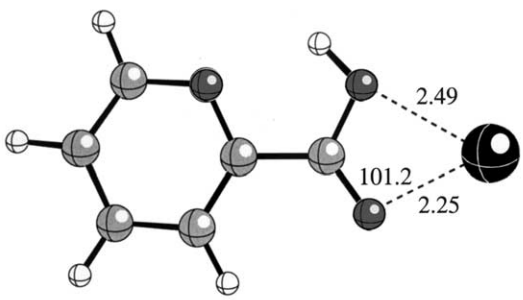


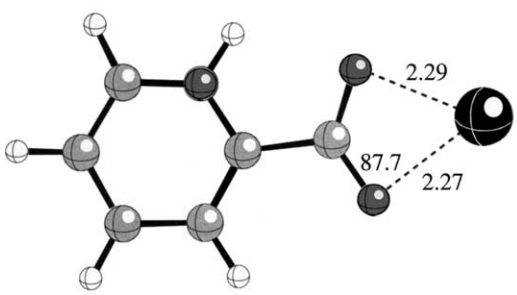
Fig. 4. Optimized geometries for isomers of the Na^+ complex of 4-HCCA at the HF/6-31G* level. Distances are in Angströms and angles in degrees.



PA-Na^+ **1**



PA-Na^+ **2**



PA-Na^+ **3**

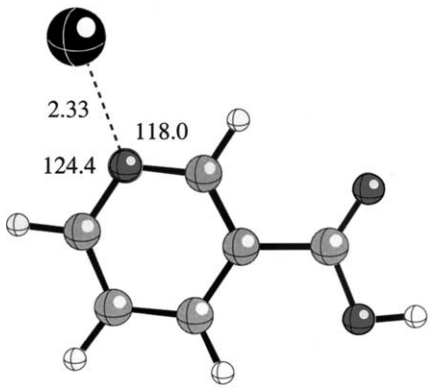
Fig. 5. Optimized geometries for isomers of the Na^+ complex of PA at the HF/6-31G∗ level. Distances are in Angströms and angles in degrees.

Since both isomers **1** and **3** are very close in energy, it might be anticipated that experimental formation of this complex will produce a mixture of these isomers. However, since there does not appear to exist a low energy pathway to interconvert both isomers, the nature of the species should strongly depend upon the experimental method used. If crystallization involves the salt bridge isomer **3** because of favorable electrostatic intermolecular interactions, then the MALDI process should form gaseous **3**, and the relevant sodium affinity in this context is that of the zwitterionic isomer of PA. The latter is computed to be higher in energy than the most stable one by 20 kcal/mol, leading to a very large complexation enthalpy of Na^+ of 61.4 kcal/mol.

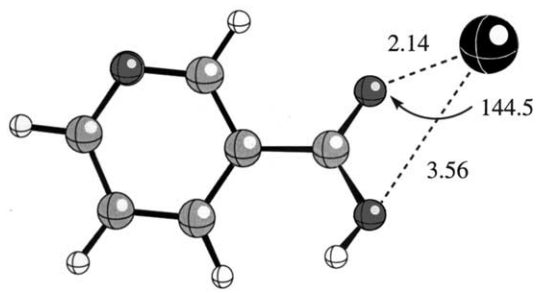
If the sodium affinity of PA is calculated from the lowest energy isomer of PA–Na^+ and the lowest energy conformer of free PA, the computed enthalpy of binding of Na^+ to PA is 42.9 kcal/mol, however, this involves PA–Na^+ **1** and a conformation of free PA analogous to that in PA–Na^+ **2**. If conservation of PA conformation upon Na^+ detachment is assumed, the binding enthalpy in **1** is worth 47.6 kcal/mol, while in **2** it is 37.8 kcal/mol.

3.5. Nicotinic acid (NA)

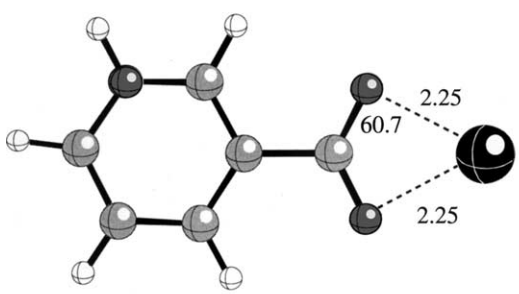
NA is an isomer of PA in which the carboxyl group is now in position 3 relative to nitrogen. The same types of isomers of the sodium complex were determined as for that of PA (see Fig. 6). A significant difference between both molecules is that now in isomer **1** the sodium ion is bound to nitrogen only, leading to a higher energy relative to other isomers. Isomer **2** has a structure somewhat different at sodium from that of isomer **2** of the PA complex because of hydrogen–hydrogen repulsion between the carboxyl and one of the ring hydrogens. It is only 1.1 kcal/mol less stable than **1** at the HF/b1 level. However, the main difference is that the salt bridge isomer **3** is now clearly the most stable of all three. A factor favoring the special stability of the salt bridge in this case, as compared to PA, is the larger dipole moment of the free acid zwitterion: 13.7 vs. 10.9 D at the MP2/b2 level. If the most stable structures of both NA–Na^+ and free NA are considered, the binding enthalpy of Na^+ to NA is computed to be 37.5 kcal/mol. In this case, this involves the salt



NA-Na$^+$ **1**



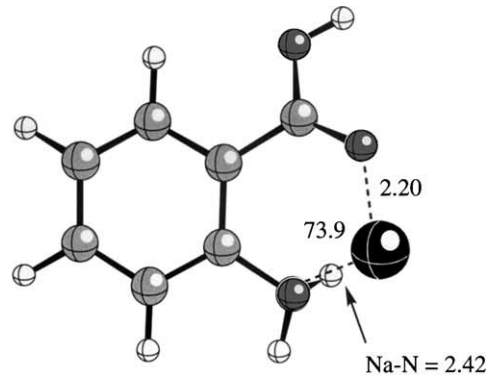
NA-Na$^+$ **2**



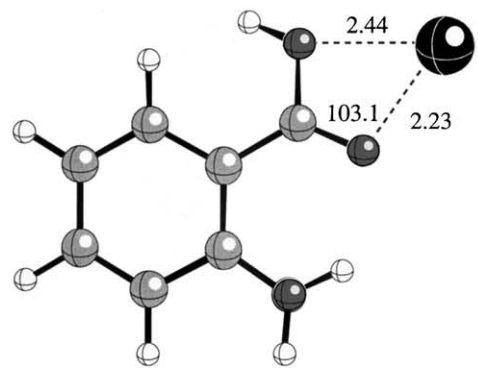
NA-Na$^+$ **3**

Fig. 6. Optimized geometries for isomers of the Na$^+$ complex of NA at the HF/6-31G∗ level. Distances are in Angströms and angles in degrees.

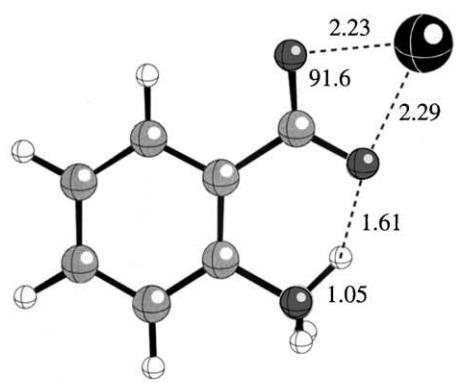
bridge isomer **3** of NA–Na$^+$ and a conformer of free NA which is structurally similar to that in NA–Na$^+$ **1**. Comparison with experiment will be done in Section 4.2.



AA-Na$^+$ **1**



AA-Na$^+$ **2**



AA-Na$^+$ **3**

Fig. 7. Optimized geometries for isomers of the Na$^+$ complex of AA at the HF/6-31G∗ level. Distances are in Angströms and angles in degrees.

3.6. Anthranillic acid (AA)

AA bears a carboxyl and an amino group in ortho positions relative to one another, therefore, it can establish efficient chelation in a pseudo six-membered ring with sodium. This structure (see **1** in Fig. 7) is the most stable isomer of the AA–Na^+ complex. In a manner analogous to those for the previous cases, two other isomers were determined: one in which the sodium ion interacts with both oxygens of the trans carboxyl group, **2**, and a salt bridge in which sodium interacts with both oxygens of the caboxylate, **3**. The latter two are close in energy, with the salt bridge slightly more stable. The relative energies of the three structures at the HF/b1 level (see Table 3) make it clear that **1** is the most stable isomer. The most stable conformation of free AA derives from that in **1** by rotating the amino group in the ring plane in order to establish a N–H···O(carbonyl) hydrogen bond. Because of this similarity, it is expected that detachment of Na^+ from AA–Na^+ **1** will produce this conformer. The associated binding enthalpy is computed to be 37.0 kcal/mol.

4. Discussion

4.1. General considerations

The sodium ion affinities calculated in this work lie in the range 34–44 kcal/mol (see Table 4). They can be compared to those for smaller molecules bearing the same functional groups. The affinity of formic acid is 23.7 kcal/mol [1], while C_1–C_4 aliphatic alcohols have binding free energies ranging from 16 to 21 kcal/mol [2,3], and small ethers have rather similar values: 17.6 and 21.3 kcal/mol for $(CH_3)_2O$ and $(C_2H_5)_2O$, respectively [2]. Aromatic compounds have also been studied, and sodium ion affinities of 21.8, 22.2 and 25.2 kcal/mol have been computed for benzene [1], phenol [1] and anisole [17]. It is clear that the affinities of the aromatic acids studied herein are significantly larger.

As has been discussed previously, the dominant attractive interactions in Na^+-molecule complexes are the electrostatic (between the ion charge and the permanent moment(s) of the molecule) and polarization components (between the ion charge and the induced moment(s) of the molecule). Polyfunctional molecules with several polar groups which can arrange in such a way as to build up a large dipole moment will lead to stronger electrostatic interactions with any given ion. Polarization terms, on the other hand, will tend to be larger for larger molecules (which are more polarizable than smaller analogues in a chemically similar series), all the more as the ion is able to approach closely several parts of the molecule. This may be achieved by conformational wrapping around the ion, leading to strong ‘solvation’. While this has been shown to be significant for aliphatic chains, and other flexible species such as peptides, the rigid nature of the aromatic ring prevents this effect from being important in MALDI matrices. Another effect potentially leading to significantly enhanced sodium ion affinity of polyfunctional molecules is chelation. The large ionic radius of sodium and its noncovalent binding makes it well suited for chelation, as opposed, for instance, to what occurs for protons. This is one of the effects explaining why there is no general correlation between sodium and proton affinities. For the six molecules considered in this work except NA, chelation leads to the most favorable isomer. It is particularly favorable when it does not disrupt hydrogen bonding as is the case for the strongest binder, 4-HCCA. For instance, as mentioned above in Section 3.1, the

Table 4
Na^+ binding enthalpies and free energies of UV-MALDI matrix molecules at 298 K, computed at the MP2/b2//HF/b1 level

Molecule	ΔH_{298}	ΔG_{298}
DHB	34.5	27.8
SA	39.0	30.7
4-HCCA	44.3	36.1
PA	42.9	35.2
NA	37.5	29.6
AA	37.0	29.0

ΔH_{298} adds a work term of RT (0.6 kcal/mol at 298 K) to the variation of internal energy at this temperature. All values are in kilocalories per mole.

sodium affinity of DHB would be 10 kcal/mol larger if hydrogen bonding did not take place in the free molecule between the carbonyl oxygen and the 2-OH group. In this hypothetical situation the sodium affinity of DHB would be roughly twice as large as those for analogous monofunctional molecules.

4.2. Comparison to experimental results

The sodium ion affinity of DHB is computed to be 34.5 kcal/mol or 144 kJ/mol. This is the binding enthalpy at 298 K, in which the lowest energy isomer of DHB–Na^+ and the lowest energy conformation of free DHB are considered. The corresponding binding free energy is 27.8 kcal/mol or 116 kJ/mol. This value may be compared to the recently obtained free binding energy of Na^+ to DHB of 158 ± 3 kJ/mol by Zenobi and coworkers [16] from ligand exchange equilibria in an FT-ICR mass spectrometer. In order to understand this rather large difference, complexation involving other isomers and/or conformers was examined. If sodium detachment from DHB–Na^+ **1** leaves the DHB molecule in the same conformation, i.e., without a hydrogen bond between the carbonyl oxygen and the 2-OH group, the enthalpy and free energy of binding increase to 44.5 and 35.8 kcal/mol or 186 and 150 kJ/mol, respectively, in much better agreement with the experimental value. If the isomer in which sodium binds to the trans acid is considered, with this conformation kept in free DHB, the ΔH and ΔG values are 42.5 and 34.4 kcal/mol or 178 and 144 kJ/mol, respectively. Therefore, it appears that the experimental result is best explained by formation of the lowest energy isomer of DHB–Na^+. The small discrepancy with the present computational results may be explained by the fact that the experimental value is derived from a sodium ion transfer equilibrium, which depends upon the accuracy of the partial pressure of DHB in the ICR cell, and upon the affinity value used for the reference base considered. It is also possible that a mixture of isomers is initially formed, in which case the affinity obtained is a weighted average of those of the various components. The present results do not point to a significant problem of this kind for DHB. Overall, it is noteworthy that the experimental conditions used do not appear to sample the lowest energy structures of *both* free DHB and its sodium complex. This implies that great care must be taken in the comparison of experimental and computational results.

The free energy of binding of Na^+ to SA is computed to be 30.7 kcal/mol or 128 kJ/mol, to be compared to a much larger experimental value of 159 ± 2 kJ/mol [16]. In this case the lowest energy conformer of free SA is maintained in the lowest energy isomer of SA–Na^+, so that this discrepancy is puzzling. If Na^+ attachment to the trans carboxyl group is considered, the binding enthalpy is further reduced by 7.5 kcal/mol. If, in the latter case, the trans conformation of the acid is assumed to be maintained after sodium detachment, a value essentially identical to the above is recovered. Thus, the discrepancy between experiment and theory remains unresolved in this case.

The free energy of binding of Na^+ to 4-HCCA is computed to be 36.1 kcal/mol or 151 kJ/mol, to be compared to an experimental value of 165 ± 3 kJ/mol [16]. Since the lowest energy conformer of free 4-HCCA is maintained in the lowest energy isomer of 4-HCCA–Na^+, the experimental and computed values are directly comparable. The agreement is reasonably good in this case.

Finally, the last case for which an experimental value of the free binding energy of sodium is available is NA [16]. Here again, a large difference exists between experiment (166 ± 1 kJ/mol) and theory (29.6 kcal/mol or 124 kJ/mol). However, in this case the comparison is not as direct as in the previous cases. The lowest energy isomer of NA–Na^+ is a zwitterion while the lowest energy isomer of free NA is neutral, and the computed value indicated in Table 4 then implies proton transfer from nitrogen to the carboxylate, concomitant with sodium ion detachment. If the zwitterion is maintained in the free NA, a very large free energy of sodium binding of 257 kJ/mol is obtained, because the zwitterion is not stabilized in the absence of the sodium ion. If, on the other hand, the lowest neutral isomer of NA–Na^+ is presumed to be involved, sodium detachment yielding

the lowest isomer of free NA requires a free energy of 22.6 kcal/mol or 94 kJ/mol. Therefore, as for SA, the discrepancy between experimental and theoretical results remains unresolved for NA. Clearly, more work is needed to clarify these issues and yield a complete picture of sodium binding to these molecules.

4.3. Comparison to corrected experimental results (added in proof)

The paper describing the experimental work cited above [16] appeared shortly after this work was reviewed. Since the procedure used to derive free energies of binding to Na^+ from experimental observations appears to warrant additional discussion, this section has been added in proof.

Sodium ion transfer equilibria or kinetics were established [16], leading to a ladder of relative free energies of binding to the sodium ion. In order to convert this relative scale into an absolute scale, a reference of known sodium affinity was chosen. The reference compound used was dimethoxyethane (DME). There exist several papers in the literature, in which the interaction of DME with Na^+ have been considered. Zhang et al. [16] chose to use the free energy of binding to Na^+ of 154 kJ/mol obtained by Castleman and coworkers [18] using high pressure mass spectrometry, although this series of values has been found to be systematically overestimated in other, more recent work [1–3,19]. Guided ion beam experiments convoluted with computed entropies and thermal corrections lead to a free binding energy of DME to Na^+ of 128.7 ± 3.9 kJ/mol [19], while sodium ion transfer equilibria between DME and several small organic molecules in an FT-ICR mass spectrometer lead to a relative scale which, when anchored with methylamine, yields a value of 133 kJ/mol [2]. The good agreement between both results leads to high confidence in suggesting that the free energy of binding of DME to Na^+ is close to 130 kJ/mol. Using this value implies a correction of all free energy values from [16], by 24 kJ/mol downwards.

The revised experimental free energy of binding of DHB to Na^+ is 134 ± 3 kJ/mol, in moderate agreement with the computed value of 116 kJ/mol. If the conformation of DHB is assumed to be retained upon sodium ion detachment, the computed free energy of binding of 150 kJ/mol is no longer in agreement with experiment. In this case, there remains a nonnegligible discrepancy between experiments and calculations. For SA, the revised experimental value of 135 ± 2 kJ/mol is now in satisfactory agreement with the computed value of 128 kJ/mol. For 4-HCCA, the revised value of 141 ± 3 kJ/mol is also in agreement with the computed value of 151 kJ/mol. Finally, the revised experimental value for NA is 142 ± 1 kJ/mol, which remains in nonnegligible disagreement with the computed value of 124 kJ/mol, although it is much less dramatic than with the nonrevised value of 166 ± 1 kJ/mol. As discussed above, the interpretation of experimental results requires caution in this case since the isomer of NA–Na^+ formed might strongly depend upon the experimental conditions used. Overall, the agreement between these revised experimentally derived and the computed free energies is much improved, although there remain open issues in some cases.

4.4. Relevance to secondary MALDI processes

What is the relevance of these results to the interpretation of MALDI processes? The latter fall into two broad categories, primary processes which occur in the condensed phase following photon absorption by solid matter, and secondary processes which occur in the gas phase (in the ‘plume’) after desorption. In the last few years, it has become increasing clear that both classes of processes have a strong influence on the observed spectra [7,10,11]. There is one result from the present work which might be relevant to the primary portion of MALDI. Crystallization of such molecules is usually considered to involve their ‘neutral’ isomer, involving no formal charge. The zwitterionic isomer of free NA is computed to be 32 kcal/mol higher in energy than its ‘neutral’ isomer at the MP2/b2//HF/b1 level. This might indeed be too large a difference for the crystal to involve zwitterions. This may be compared to amino acids which are zwitterions in the solid state. The energy difference between isomers varies

largely from one amino acid to another, with a large value of ca. 17 kcal/mol for glycine. Still this is much lower than the value computed for NA. On the other hand, adding a sodium salt leads to a salt bridge being most stable for gaseous NA–Na^+, and this is likely to carry over to the solid state with strong intermolecular attractions, even if crystallization involves distal counterions. This may favor the direct desorption of preformed matrix–Na^+ ions with a salt bridge structure.

The present results may otherwise be used to make progress in the evaluation of secondary processes, plume chemistry. The sodium ion affinities determined herein are large, but still smaller than what is expected for biomolecules such as peptides. For instance the affinity determined for the simplest peptide, glycylglycine, is 45 kcal/mol [4a], and larger peptides are expected to have significantly larger affinities. Therefore, the direct sodium transfer reaction from the matrix to the analyte molecule is expected to be exothermic in such cases, leading to the efficient formation of sodiated analytes.

Another general process which has been invoked in the plume is the interconversion of matrix–Na^+ and matrix–H^+ species [11]:

$$\text{matrix–Na}^+ + \text{matrix} \rightarrow \text{matrix–H}^+ + [\text{matrix} - \text{H}]\text{Na} \quad (1)$$

Such processes may be important since it is well known that different matrices exhibit different tendencies to appear as protonated or sodiated in MALDI mass spectra [9] (of course, other reactions should be coupled to reaction 1 in order to understand the observed intensities, such as proton and sodium ion transfer between analyte and matrix molecules).

As discussed by Knochenmuss et al. [11], reaction 1 may be conveniently split into three steps:

$$\text{matrix} + \text{matrix} \rightarrow \text{matrix–H}^+ + [\text{matrix} - \text{H}]^- \quad (1a)$$

$$\text{matrix} - \text{Na}^+ \rightarrow \text{matrix} + \text{Na}^+ \quad (1b)$$

$$[\text{matrix} - \text{H}]^- + \text{Na}^+ \rightarrow [\text{matrix} - \text{H}]\text{Na} \quad (1c)$$

Reaction 1b outlines the importance of the sodium affinity of the matrix molecule, however, the present results show that variations are in a relatively limited range and thus, the trend might well be counterbalanced by the variation of other terms. In order to determine the energy associated with reaction 1, we have computed the deprotonated forms of NA and of its sodium complex. Results at the MP2/b2//HF/b1 level show that the 298 K enthalpy of reaction 1c is 137 kcal/mol. A value of 143 kcal/mol (quoted as 6.2 eV) has been computed for reaction 1c in the case of DHB [11]. The enthalpy of reaction 1a is known for several matrix molecules, including NA (118 kcal/mol) and DHB (121–123 kcal/mol; see Table 3 of [7]) [20–22]. Thus, it appears that for each of the three steps 1a–1c, there is no large difference in the variation of enthalpy between DHB and NA. The overall enthalpy of reaction 1 is unfavorable by 20 kcal/mol for NA and by 12 kcal/mol for DHB. Thus, this type of scenario argues in favor of sodiated, rather than protonated matrices as charge sinks in ion–molecule chemistry in the plume. It would be interesting to extend such calculations to a range of other matrices to see if other cases might also favor the sodiated form.

5. Conclusion

Accurate enthalpies and free energies of binding to the sodium ion in the gas phase have been determined by ab initio calculations for six aromatic acids commonly used as matrices in MALDI. The enthalpies of sodium binding range from 33 to 44 kcal/mol. The most stable isomers of the sodium complexes frequently involve chelation of sodium between functional groups, and in one case, NA, a salt bridge is the most favorable structure. Comparison with recently determined experimental free energies of binding suggests that, if the latter are revised to improve their absolute anchoring, satisfactory agreement exists for SA and 4-HCCA, while significant differences remain for DHB and NA. More work appears to be needed to resolve these issues.

The gaseous Na^+ affinities determined herein may be used as input to evaluate the relevance of recently

proposed scenarios of ion–molecule chemistry in the MALDI plume. Interconversion between protonated and sodiated matrix molecules is found to favor the sodiated form for NA and DHB.

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